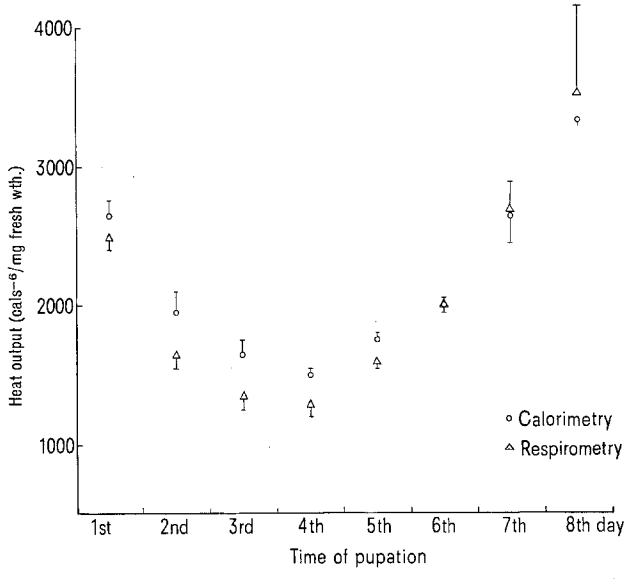
large proportion of the total heat produced, particularly in poikilotherms, it is clear that considerable error can be introduced into the measurements. Indeed, calorimetry has tended to be discounted as an alternative to respirometry for this reason (PETRUSEWICZ and MACFADYEN[2], SOUTHWOOD[4]).

This note is a preliminary account of a comparison between respirometry and calorimetry in measuring the metabolic energy costs of pupation in *Tenebrio molitor* L. The results are summarized in the Table, where the heat output estimated by calorimetry has been corrected for the heat absorbed by evaporation and the heat output estimated by respirometry has been arrived at by multiplying oxygen consumption by IVLEV's[5] oxycalorific equivalent. When heat output is plotted against time (Figure) the familiar U-shaped curve results, and, futhermore, it will be seen that the 2 methods produce very similar curves. Calorimetry apparently gives a slightly higher estimate for the first 5 days of pupation but respirometry does so in the last 3 days. However, at no time are these differences significant. It can be concluded, therefore, that, if proper allowance is made for the heat absorbed by evaporation, calorimetry provides as good a measure of the energy dissipated in metabolic processes as respirometry.



The metabolic energy costs of pupation in *Tenebrio molitor* L.: A comparison between calorimetry and respirometry.

These results can be interpreted in two ways. Firstly, they may be regarded as confirmation that respirometry is a satisfactory method, but it should be remembered that, while *T. molitor* pupae are well adapted to the conditions obtained in the respirometer, many poikilotherms are not and it would be unwise to assume that the results can be extended to cover them all. Alternatively, it can be argued that calorimetry is a viable method which, besides having the advantage of giving a direct measure of energy dissipation, is theoretically capable, unlike the majority of respirometric methods, of accommodating the experimental animals in an environment close to that in which they normally live. Since many of the poikilotherms of interest in production studies are found conditions of high humidity, avoiding the physiological stresses attendant on desiccation, establishment of natural conditions within the calorimeter will have the additional advantage of reducing the correction necessary for the latent heat of vaporization. The recently described modification to the LKB Flow Microcalorimeter (ERIKSSON and WADSÖ[6]), which allows of the oxygenation of the contents of the reaction vessel, suggests that it is not only theoretically, but also practically, possible to establish an atmosphere congenial to any experimental animal. For these reasons calorimetry deserves more favourable consideration as an alternative method for estimating metabolic costs.

A fuller account of these experiments and the methods employed will appear elsewhere[7].

Zusammenfassung. Durch Vergleich respirometrisch und kalorimetrisch bestimmter Daten über den Energieverbrauch während der Verpuppung von *Tenebrio molitor* wird gezeigt, dass die letztgenannte Methode der ersteren ebenbürtig ist, bei feuchtigkeitsliebenden Arten sogar überlegen sein müsste.

G. J. PEAKIN[7]

Biological Sciences Department, Goldsmiths College, London, S.E.14 6N.W. (England), 29 December 1972.

[4] T. R. E. SOUTHWOOD, *Ecological Methods* (Methuen, London 1966).
[5] V. G. IVLEV, Biochem. Z. *275*, 49 (1934).
[6] R. ERIKSSON and I. WADSÖ, Proc. 1st Eur. Biophys. Congr. (1971), vol. 4, p. 319.
[7] I am most grateful to LKB-Produkter AB., S-161125 Bromma 1, Sweden and their staff for the extended loan of a modified Batch Microcalorimeter.

The Influence of Hydrostatic Pressure on the Rate of Hydrolysis of Acetylcholine and Contractility in the Vagal Heart System

Previously we have shown that hydrostatic pressure blocks the vagal inhibition completely at 5,000 ψ[1]. Our value is considerably lower than that reported by CATTELL and EDWARDS[2], BROWN[3] and EDWARDS and BROWN[4] for inhibition of muscular contraction in the skeletal muscles. We further demonstrated that physostigmine, a specific acetylcholinesterase inhibitor, raises the blocking pressure from 5,000 to 6,500 ψ. We concluded that the effect of hydrostatic pressure on the vagal inhibition is primarily a conformational change of acetylcholinesterase molecule located at the post-junctional membrane.

Over the last decade we have examined rather extensively the activity of this particular in situ enzyme with respect to various physical[1,5,6] and chemical[7–9] parameters. This report is a further investigation on the rate of hydrolysis of acetylcholine by acetylcholinesterase and the extent of volume change in the enzyme molecule as a function of pressure.

Materials and methods. The experiments were performed on isolated vagal heart preparation of frogs, *Rana pipiens*, The heart was perfused with modified Ringer's solution, which has the following composition: NaCl,

112 mM; KCl, 2 mM; $CaCl_2$, 2 mM; NaH_2PO_4, 0.1 mM and $NaHCO_3$, 1.8 mM.

The recording system has been described in detail elsewhere[5]. The regular moist chamber was replaced by a high pressure chamber. The stainless steel chamber was 3 cm in diameter and 10 cm in height. 2 quartz windows, 1.5 cm thick, served as a light pathway, the intensity of which was registered via a phototube. A pair of platinum electrodes passing through the lid was fitted with special high pressure fittings and insulated with epoxy. The high pressure system is shown in Figure 1.

The contractility was determined by the height of the tracings, which are proportional to the force of contraction. Quantitative measurements of the force of cardiac contraction were possible by transmission of the electrical signal from the phototube to an integrating digital voltmeter. The acetylcholinesterase activity was determined according to the method described previously[7].

Calculation of the volume change of the in situ enzyme under pressure. Using the Michaelis-Menten formulation[5]

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E.S \xrightarrow{k_3} E + B \qquad (1)$$

where k_1, k_2 are reaction constants and k_3 is the constant for decomposition of enzyme-substrate complex. E is the enzyme, acetylcholinesterase; S is the substrate, acetylcholine; B is the product. E.S is a complex in which the enzyme and substrate are held together by VAN DER WAALS forces, dipole-dipole interactions and electrostatic bindings[6]. Since the substrate is in large excess by sudden electrical pulsing release, then assumption of a steady state concentration of complex E.S leads to the rate equation:

$$d(B)/dt = k_3 K (E_o)\ (S)/(1 + K(S)) \qquad (2)$$

where K is the ratio $k_1/(k_2 + k_3)$ and E_o is the total enzyme concentration.

Since an apparent volume of activation is defined by the relation

$$\Delta V^* = -RT\partial 1u\ (d(B)/dt)/\partial p. \qquad (3)$$

A convenient form of this relation for computing the value ΔV^* from rates measured at different pressures is

$$\Delta V^* = -2.303\ RT \log (k_{p_2}/k_{p_1})/(p_2 - p_1) \qquad (4)$$

where R is the gas constant; T, the absolute temperature; k_{p_1} and k_{p_2} are the rate constants of the enzyme at pressures p_1 and p_2 respectively.

An example of computation. The experimental rate ratio is 1.98, p_1 and p_2 are 14.7 ψ and 3,000 ψ or 1 atm. and 204 atm. R equals 0.082 l atm/mol, and T si 293 °K.

$$\Delta V^* = -(2.3)\ (0.082)\ (293)\ (0.299)/203$$

$$\Delta V^* = -0.0815 \text{ l/mol} = -81.5 \text{ cm}^3\text{/mol}.$$

Results and discussions. Figure 2 shows the actual tracing under normal and high pressures. The quantitative relationship between hydrostatic pressure and the rate of acetylcholine hydrolysis is shown in Figure 3. There has been no appreciable change in the rate of hydrolysis below 2,000 ψ. The rate of hydrolysis increases steadily as the pressure increases and reaches maximum at 5,000 ψ. As indicated in the Figure, the rate at 5,000 ψ is more than 4-folds of the original rate at 14.7 ψ, namely the atmospheric pressure.

The contractility of the isolated heart as a function of pressure is shown in Figure 4. There are 7%, 27%, 44%,

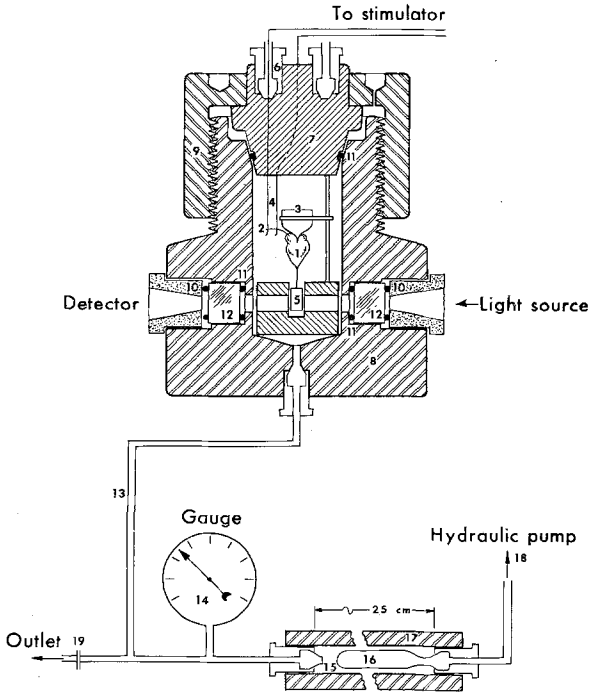


Fig. 1. High pressure system. 1, heart; 2, vagus nerve; 3, modified Straubs cannula; 4, platinum electrodes; 5, weight; 6, pressure fittings; 7, cover of the chamber; 8, stainless steel chamber; 9, clamping ring; 10, brass window retainer; 11, '0' ring gasket; 12, quartz window; 13, connecting high pressure tubing; 14, pressure; 15, interior of the accessory chamber communicating to the main chamber; 16, Tygon sac, acting as a diaphragm to prevent oil from contaminating main pressure chamber which was filled with Ringer's solution; 17, wall of accessory chamber; 18, outlet to hydraulic pump; 19, outlet to atmosphere.

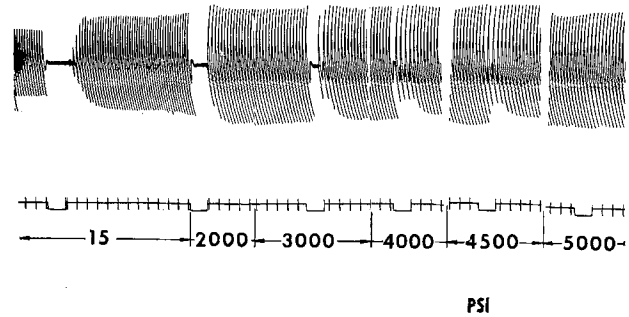


Fig. 2. Effect of pressure on vagal inhibition. A, 15 ψ; B, 2.000 ψ; C, 3.000 ψ; D, 4.000 ψ; E, 4.500 ψ; F. 5.000 ψ.

[1] W. YOUNG and F. UPHAM, Am. J. Physiol. *202*, 947 (1962).
[2] MCK. CATTELL and D. J. EDWARDS, Am. J. Physiol. *93*, 97 (1930).
[3] D. E. BROWN, Cold Spring Harb. Symp. quant. Biol. *4*, 242 (1936).
[4] D. J. EDWARDS and D. E. BROWN, J. cell. comp. Physiol. *5*, 1 (1934).
[5] W. YOUNG, *Magnetic Field and in situ Acetylcholinesterase in the vagal Heart System. In Biological Effects of Magnetic Field.* (Ed. F. M. BARNOTHY; Plenum, New York 1969).
[6] W. YOUNG, Experientia *26*, 592 (1970).
[7] W. YOUNG and J. W. GOFMAN, Biochim. biophys. acta *64*, 60 (1962).
[8] W. YOUNG, UCRL *14739*, 97 (1966).
[9] W. YOUNG, Physiologist *14*, 258 (1971).

Net volume changes computed according to observed influence of pressure on specific enzyme reactions in vitro and in situ

Enzyme	Substrate	Pressure (ψ)	Concentration (M + %)	Temperature (°C)	Observed vol. change (cm^3/mol)	References
Acetylcholinesterase (in situ)	Acetylcholine	2.000	10^{-8}	20	— 49.5	this work
		3.000	10^{-8}	20	— 81.5	
		4.000	10^{-8}	20	—104.0	
		4.500	10^{-8}	20	—110.0	
		5.000	10^{-8}	20	—108.1	
		6.000	10^{-8}	20	— 91.5	
Amylase	Starch	10.000	1	22–23	—22	BENTHAUS[10]
Invertase	Sucrose	10.000	10	30	—69	EYRING, JOHNSON and GENSLER[11]
Pancreatic amylase	Starch	10.000	2	22–23	—28	LAIDLER[12]
Chymotrypsin	Casein	10.000	1	14.8	—13	WERBIN and MCLAREN[13]
Trypsin (in vitro)	β-Lactoglobulin	10.000	0.5	25–35	—36	WERBIN and MCLAREN[13]

61%, 67% and 58% increase in contractility when the preparation exposed to hydrostatic pressure of 1,000 ψ, 2,000 ψ, 3,000 ψ, 4,000 ψ, 5,000 ψ and 6,000 ψ respectively. The tension of turtle heart under similar pressure reported by BROWN[3] is also shown in Figure 4 for comparison. In general our value is very close to that of BROWN's, especially at the lower pressure range, i.e. below 2,000 ψ. At higher pressure range: 4,000 ψ, 5,000 ψ, 6,000 ψ, our value seems to be higher. This may be explained by the fact that we measure the force change rather than tenison.

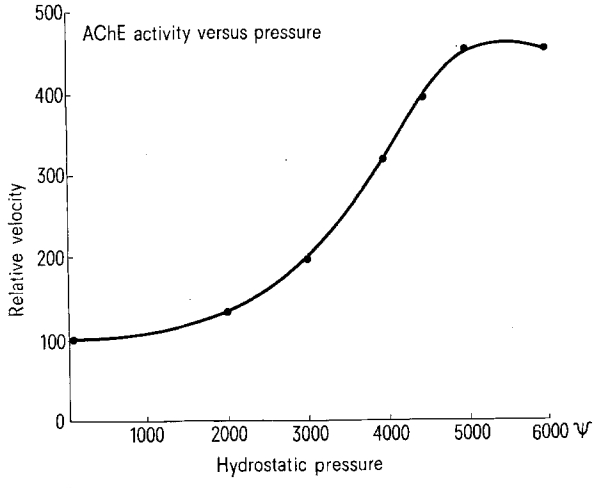


Fig. 3. The effect of pressure on acetylcholinesterase activity.

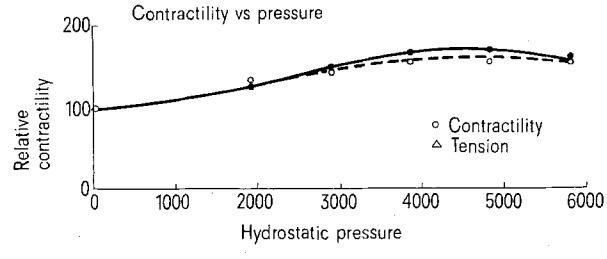


Fig. 4. The effect of pressure on contractility of the isolated frog's heart and the tension of turtle's heart. ○—○, contractility of frog's heart; △---△, the tension of turle's heart.

A computation of the molecular change under different pressure is shown in the Table. The values of ΔV^* calculated according to equation 4 from our experiment are in line with those reported in the in vitro enzyme experiment. The values for the in situ acetylcholinesterase are higher than that of the in situ enzyme values. It is tempted to interpret that the enzyme molecules used for the in vitro experiments are denatured during the process of isolation. Therefore the sensitivity of the in vitro enzymes to pressure is decreased to a certain extent.

The change in volume of the enzyme is significant for the disengaging of the electro-coupling of the heart contraction. A calculation of the volume change per enzyme molecule reveals that each molecule shrinks about 2.5 Å, if the 3 axes of the enzyme molecule are equal. If the 2 short axes are equal and the long axis is 20 times the short axes, then the molecule would shrink about 6.7 Å along the long axis assuming the molecule shrunk uniformly in all directions. On the basis that each molecule of the enzyme has 4 active sites, then the distance between the active sites would probably shrink about 0.64 Å and 1.87 Å for $x = y = z$, and $20x = 20y = z$ respectively. X, Y and Z are the 3 axes. This finding indicates that the change in distance between the active sites produced by pressure may be responsible for the observed acetylcholinesterase activity in this study.

Zusammenfassung. Die Acetylcholin-Esterase-Aktivität und die Kontraktilität wird am isolierten Frosch-Herzen unter steigendem hydrostatischem Druck untersucht. Ohne wesentliche Veränderung der Molekül-Dimension wird ein Anstieg der Hydrolyse von Acetylcholin bewirkt.

W. YOUNG[14, 15]

Lawrence Radiation Laboratory, University of California, Berkeley (California, USA), 19 January 1973.

[10] J. BENTHAUS, Biochem. Z. *311*, 108 (1941).
[11] H. EYRING, F. H. JOHNSON and H. L. GENSLER, J. phys. Chem., Ithaca *50*, 453 (1946),
[12] K. J. LAIDLER, Archs Biochem. Biophys. *31*, 285 (1961).
[13] H. WERBIN and A. D. MCLAREN, Arch. Biochem. Biophys. *31*, 417 (1951).
[14] National Academy of Sciences, National Research Council, Senior postdoctoral Resident Research associate.
[15] Present address: 223-6, Ames Research Center, NASA, Moffett Field, California 94035, USA.